

*The fifth Datta Lecture***Structural similarities between the aspartate receptor of bacterial chemotaxis and the *trp* repressor of *E. coli*****Implications for transmembrane signaling****Berkley A. Lynch\* and Daniel E. Koshland Jr.***Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720, USA*

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A high resolution structure of the N-terminal ligand-binding domain of the aspartate receptor which mediates aspartate chemotaxis in *Salmonella typhimurium* has recently been reported. A least-squares superposition of the  $\alpha$ -amino nitrogen,  $\alpha$ -carbon,  $\beta$ -carbon, and  $\alpha$ -carboxylate carbon of the aspartate bound to the aspartate receptor onto the equivalent atoms in the tryptophan bound to the *trp* repressor provides evidence for similarity between key parts of the active sites that bind to the  $\alpha$ -amino and  $\alpha$ -carboxylates of the respective ligands. Because the N-terminal domain of the aspartate receptor and the *trp* repressor also share other structural similarities, we hypothesize that the similarity between the aspartate receptor and the *trp* repressor derives from a similarity in ligand-induced conformational changes at the active sites of these proteins. This hypothesis also implies that an important signaling event in the aspartate receptor occurs through tertiary conformational changes within a single subunit.

Aspartate receptor; Transmembrane signaling; Tryptophan repressor; Chemotaxis

**1. INTRODUCTION**

A number of cell-surface receptors have been cloned and sequenced, and have been classified into different groups based on the number and arrangement of their hydrophobic transmembrane regions. For example, the chemotaxis-system aspartate receptor from *Escherichia coli* and *Salmonella typhimurium* is a member of the class of receptors which contain one or two hydrophobic transmembrane regions, as is the insulin receptor and the epidermal growth-factor receptor. The aspartate receptor is studied in our laboratory in order to understand how a transmembrane signal is generated, with the hope that this system will prove to be a model for transmembrane signaling in other receptors.

The aspartate receptor has an N-terminal periplasmic ligand-binding domain, a C-terminal cytoplasmic signaling domain, and two transmembrane regions [1]. The

aspartate receptor is a dimer of identical subunits. Detergent-solubilized receptor is dimeric in both the presence and absence of aspartate [2]. A high resolution structure of the *S. typhimurium* aspartate receptor N-terminal periplasmic ligand-binding domain (AR-N) has been solved to atomic resolution [3]. This fragment ends at the beginning of the transmembrane regions. Cross-linking studies of the transmembrane domain of the aspartate receptor allowed mapping of interactions between transmembrane helices [4]. The combination of the cross-linking data and the crystal structure allowed the development of a model containing the transmembrane helices of the receptor combined with the N-terminal, ligand-binding domain of the receptor [3].

There is currently contradictory evidence about the changes which occur on binding of aspartate to the receptor, with some crystallographic evidence favoring an intersubunit quaternary mechanism [3], and some biochemical evidence favoring an intrasubunit tertiary mechanism [4,5]. In an attempt to understand better the conformational changes that occur in the aspartate receptor during signaling, we have compared the aspartate binding site of the AR-N domain with the binding site of the *trp* repressor of *E. coli*. The tryptophan repressor is a transcriptional factor that controls expression of genes in the tryptophan operon. Binding of tryptophan activates the *trp* repressor, increasing its specific affinity for DNA [6,7].

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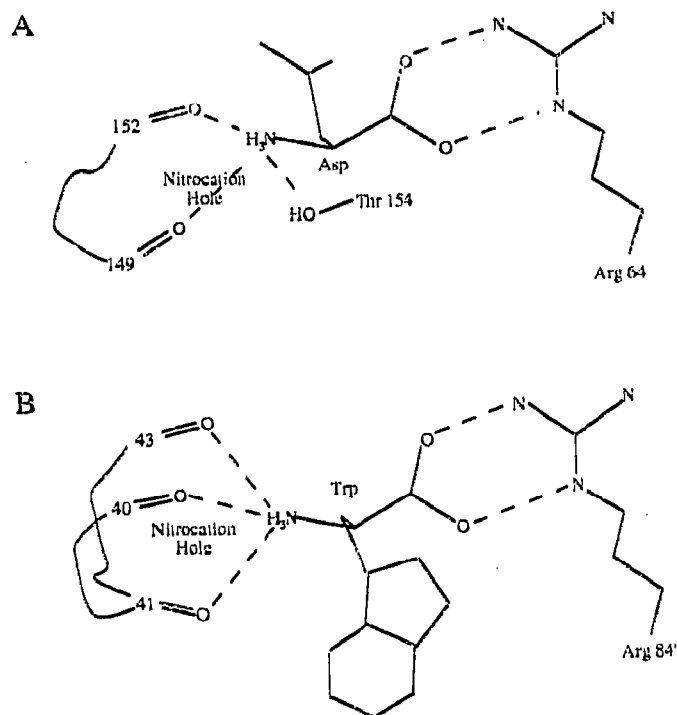


Fig. 1. Ligand-binding sites of the aspartate receptor N-terminal domain with bound aspartate, and the *trp* repressor with bound tryptophan are diagramed. Dotted lines represent potential hydrogen bonds. (A). The ligand-binding site of the aspartate receptor showing the arginine residue which interacts with the  $\alpha$ -carboxylate group of the aspartate ligand, as well as the backbone carbonyl oxygens and threonine hydroxyl which form the nitrocarbon hole and hydrogen bond to the  $\alpha$ -amino atoms of the bound aspartate. (B). A similar representation of the ligand-binding site of the *trp* repressor.

The aspartate receptor and the *trp* repressor show similarities in their ligand-binding sites. In this study we attempt to formulate a functional basis for the binding site similarities between these proteins. Our analysis leads us to propose that the similarities exist because of similar conformational changes that occur in the two proteins on binding or release of their amino acid ligands. We will argue that the conformational change occurring in the aspartate binding site of the aspartate receptor during signaling resembles local conformational changes that occur in the ligand-binding site of the *trp* repressor.

## 2. MATERIALS AND METHODS

Comparisons between the N-terminal domain of the aspartate receptor of chemotaxis and other protein crystal structures were done by rotating and translating the receptor into the unit cell of each of the other compared proteins, using the best fit superposition between atoms in the aspartate ligand of AR-N and the equivalent atoms in the ligand of the protein of interest. All studies were done using the Quanta software package (Polygen

Corp. Redwood City, CA). Identical sequence positions in equivalent subunits in oligomeric proteins are denoted as *n* and *n'*, and interactions between two equivalent regions on different subunits in the dimer are referred to as interactions between regions *a* and *a'* (for instance helix 1 in one subunit, and helix 1' in the other, identical subunit). The two transmembrane helices in the aspartate receptor are referred to as helices TM1 and TM2, while the helices which lead into them in the 4-helix bundle of the periplasmic domain, AR-N, are referred to as  $\alpha$ 1 and  $\alpha$ 4, respectively. While structures have been obtained of both liganded and unliganded forms of AR-N, the differences between the two are very small, so all comparisons are done with the liganded form of the receptor [3].

Coordinates of the *trp* aporepressor (entry 3WRP, version of 12/87) and the complex with tryptophan (entry 2WRP, version of 12/87) [8] were obtained from the protein Data Bank at Brookhaven National Laboratory [9,10]. Detailed comparisons between AR-N and the *trp* repressor were generated by superimposing the ligands through calculating a least-squares fit of the  $\alpha$ -amino,  $\alpha$ -carbon,  $\alpha$ -carboxylate carbon, and  $\beta$ -carbon of the aspartate bound to AR-N and the tryptophan bound to the repressor. To compare the proteins AR-N and the *trp* aporepressor, a least-squares fit superposition was calculated using the backbone atoms of residues 20 to 60 in the aporepressor to the same residues in the *trp* repressor, which had already been superimposed on the AR-N protein. This region exhibits minimal tertiary changes between the *trp* repressor and aporepressor [11].

The binding sites of other proteins with bound amino-acid ligands were also examined for their similarities to the aspartate receptor. Additional proteins we looked at are the leucine/isoleucine/valine-binding protein (LIVBP; entry 2LIV, version of 4/89) [12], and aspartate transcarbamylase (ATCase) with bound *N*-phosphonyl acetyl-aspartate (PALA) (entry 8ATC, version of 8/89) [13] (both from *E. coli*, and both obtained from the Protein Data Bank at Brookhaven National Laboratory). We also examined aspartate amino-transferase (AATase), with bound  $\alpha$ -methyl aspartate, from chicken mitochondria (J.N. Jansonius, personal communication).

## 3. RESULTS

The  $\alpha$ -carboxylate and  $\alpha$ -amino groups of the respective ligands of AR-N and the *trp* repressor are bound in almost identical ways (Fig. 1A and B). AR-N binds the  $\alpha$ -amino atom of bound aspartate using hydrogen bonds with two backbone carbonyls, from residues 149 and 152, which form part of a bulge in helix  $\alpha$ 4. The side-chain hydroxyl of Thr-154 is also oriented to make a hydrogen bond with the  $\alpha$ -amino atom of the aspartate ligand. The *trp* repressor binds the  $\alpha$ -amino group

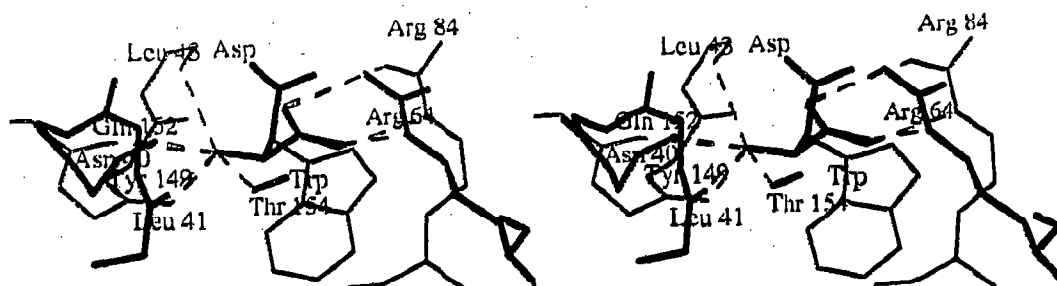


Fig. 2. Superposition of the ligand-binding sites of the *trp* repressor complexed with tryptophan and AR-N complexed with aspartate. Heavy lines are the bonds in the aspartate receptor, light lines are those in the *trp* repressor, and dashed lines are potential hydrogen bonds. Shown are the superimposed ligands, aspartate and tryptophan; the backbone atoms of residues containing carbonyl oxygens which hydrogen bond to the ligand amino nitrogens; and residues Arg-64 in AR-N, Arg-84' in the *trp* repressor, and the Thr-154 sidechain hydroxyl in AR-N. The binding sites were superimposed by a least-squares fit on the  $\alpha$ -amino nitrogen,  $\alpha$ -carbon,  $\alpha$ -carboxylate carbon, and  $\beta$ -carbon of the ligands of each protein.

of its ligand, tryptophan, using hydrogen bonds to backbone carbonyls from three residues: 40, 41, and 43, which lie at the N terminus of helix B. This 'carbonyl pocket' is reminiscent of the oxyanion hole seen in serine proteases [14,15]. In serine proteases, backbone amide hydrogens hydrogen bond to the amide oxygen of the substrate, and are thought to stabilize the oxygen in a negatively charged state, as part of the tetrahedral form of the transition state intermediate. In AR-N and the *trp* repressor, the equivalent relationship is oxygens from backbone carbonyls, and a side-chain hydroxyl in AR-N, interacting with a positively charged amino group of the ligand. This is almost a mirror-image of the oxyanion hole, and in an equivalent fashion would be termed the nitroocation hole.

AR-N and the *trp* repressor also bind the  $\alpha$ -carboxylates of their respective ligands in similar ways. AR-N binds the  $\alpha$ -carboxylate of aspartate, using a salt bridge with the side-chain guanidinium of arginine residue 64 (Fig. 1A). In the tryptophan repressor, the  $\alpha$ -carboxylate of the bound tryptophan ligand forms a salt bridge with arginine residue 84' (Fig. 1B). One difference between the two proteins is that in AR-N residue 64 and residues 149, 152, and 154 are from the same subunit, while in the *trp* repressor the equivalent residues 84' and 40, 41 and 43, come from different subunits.

Superimposing the  $\alpha$ -carbon,  $\beta$ -carbon,  $\alpha$ -carboxylate carbon, and  $\alpha$ -amino groups of the two ligands, aspartate and tryptophan, bound to their respective proteins, using a least-squares procedure, demonstrates that these atoms of the two ligands could be overlaid almost exactly (rms = 0.05). The two ligands differ substantially in the orientation of the remainder of their structures (the  $\beta$ -carboxylate of aspartate, and the indole ring of tryptophan) (Fig. 2). The ligand superposition reveals a corresponding spatial juxtaposition of Arg-64 in AR-N with Arg-84' in the *trp* repressor (Fig. 2). Both of these residues coordinate with the  $\alpha$ -carboxylate of their respective ligands in almost exactly the same way, with equivalent nitrogen atoms from the two

residues lying from one to three Angstroms apart. In both cases, a terminal nitrogen and the Nz nitrogen from the guanidinium groups of the Arg residues interact with the  $\alpha$ -carboxylate oxygens of the amino-acid ligands.

The superposition demonstrates that the relative locations and orientations of the backbone carbonyl groups, that interact with the ligand  $\alpha$ -amino groups, are similar in the two proteins. The backbone carbonyls from the overlaid proteins that coordinate to the ligand  $\alpha$ -amino atoms lie in an equivalent orientation to the ligand  $\alpha$ -amino atoms, even though only two of the carbonyls, from residue 149 in AR-N and residue 41 in the *trp* repressor, are closer than 1 Å in space in the superimposed structures (within 5 Å distance). As a result of their similar orientations, hydrogen bonds can be calculated to form between the  $\alpha$ -amino group of either ligand and the carbonyls from the other protein in the superimposed structures. Arg-64 in AR-N and Arg-84' in the *trp* repressor are both found in  $\alpha$ -helices. The backbone carbonyls from both proteins that interact with the ligand  $\alpha$ -amino atoms derive from residues at the termini of  $\alpha$ -helices.

Crystal structures of the *trp* repressor have been solved with the tryptophan ligand bound and absent. In the absence of ligand, the *trp* aporepressor is conformationally constrained from binding to its specific DNA recognition site. Superimposing the *trp* aporepressor onto the *trp* repressor structure that had previously been superimposed onto the AR-N structure, allows a comparison between the aporepressor and AR-N structures. A superposition of the AR-N and *trp* aporepressor ligand-binding sites (Fig. 3) shows that Arg-84', which had formed a salt bridge with the  $\alpha$ -carboxylate of the tryptophan ligand, swings in to occupy the space vacated by the ligand. In this position a terminal nitrogen atom from Arg-84' interacts with the same backbone carbonyls of the protein as the  $\alpha$ -amino group of tryptophan in the ligand-bound repressor. As a result of the superposition, Arg-84' is in a position where, if

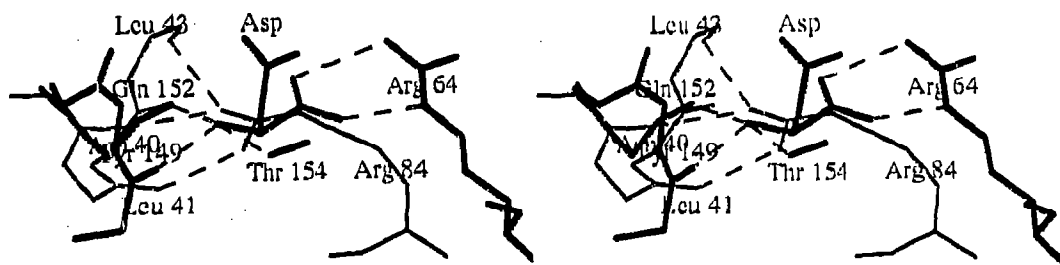


Fig. 3. Superposition of the ligand-binding sites of the apo form of the *trp* repressor with the complex form of the aspartate receptor. To generate this overlay, the complex form of the *trp* repressor was first overlaid on the complex form of the aspartate receptor as in Fig. 2. Next, the apo form of the *trp* repressor was overlaid on the complex form of the *trp* repressor, using a least-squares fit between residues that do not change significantly between the two structures (see materials and methods). Heavy lines are the aspartate receptor and its bound aspartate ligand. Light lines are the *trp* aporepressor. Dashed lines are potential hydrogen bonds. Shown are the ligand aspartate; the backbone atoms of residues containing carbonyl oxygens which hydrogen bond to the ligand amino nitrogens; and residue Arg-64 in AR-N, Arg-84' in the *trp* repressor, and the Thr-154 sidechain hydroxyl in AR-N. Note that Arg-84 in the *trp* aporepressor overlaid on AR-N occupies the position of the bound aspartate, with one of its terminal nitrogens almost exactly overlaid on the aspartate  $\alpha$ -amino group.

it were in the same protein, it could hydrogen bond with the oxygen atoms in AR-N which hydrogen bond to the  $\alpha$ -amino group of the aspartate ligand.

Two of the other amino-acid binding proteins, AATase and LIVBP, share little active site similarity to the aspartate receptor. In the case of LIVBP, there are no arginines interacting with the  $\alpha$ -carboxylate of the leucine ligand, although the  $\alpha$ -amino atom of bound leucine ligand interacts with a backbone carbonyl in the protein. AATase has several arginine residues that interact with bound  $\alpha$ -methyl aspartate substrate analogue, but they all interact in a different geometrical orientation than that seen in the aspartate receptor and the *trp* repressor. These arginines interact via the two terminal guanidinium nitrogen atoms (an 'end on' orientation), rather than via one terminal nitrogen and the Nz nitrogen (a 'sideways' orientation). There are no interactions between backbone carbonyls and the  $\alpha$ -amino group of  $\alpha$ -methyl aspartate.

The final protein examined, ATCase, did show some binding-site similarity with the aspartate receptor and the *trp* repressor. ATCase is an allosteric enzyme, and undergoes ligand-induced conformational changes in its active site. ATCase has an active site arginine which interacts in a 'sideways' manner with the  $\alpha$ -carboxylate-equivalent of the bound substrate analogue PALA. There is no nitrocatation hole structure in the active site of ATCase; instead there is a single backbone carbonyl which interacts with the PALA ligand. Despite some similarities with the aspartate receptor, we think that the *trp* repressor is the better model for understanding conformational changes in the aspartate receptor. One key difference between ATCase and the aspartate receptor is that the region of the enzyme that interacts with the  $\alpha$ -amino equivalent atom of PALA is quite different from the *trp* repressor and the aspartate receptor. (As mentioned, ATCase lacks a nitrocatation hole structure.) In addition, the active site of ATCase is not composed

of interacting  $\alpha$ -helices, unlike the aspartate receptor and *trp* repressor. Finally, ATCase is a large multi-subunit enzyme. Both the aspartate receptor N-terminal domain and *trp* repressor are smaller, dimeric proteins composed predominantly of  $\alpha$ -helical secondary structure.

#### 4. DISCUSSION

Surveying available protein structures with amino acid ligands bound, we immediately noticed strong similarities between the aspartate receptor N-terminal domain and the tryptophan repressor. The *trp* repressor and AR-N both bind amino acid ligands, and undergo conformational changes on binding these ligands. Both protein structures are almost completely  $\alpha$ -helical, and are dimeric, with amino acid ligands bound at their subunit interfaces. The binding sites in both proteins lie between two anti-parallel  $\alpha$ -helices, one of which contains an arginine residue, and the other of which contains the nitrocatation hole. In both proteins the orientation and interactions of the oxygens of the nitrocatation holes with the  $\alpha$ -amino groups of the ligands are very similar, and both proteins have arginine residues that interact in almost identical ways with the  $\alpha$ -carboxylates of the bound ligands. None of the other proteins examined have the combination of a nitrocatation hole and an active site arginine interacting in the described geometry. Additionally, none of the other proteins have active sites that are composed largely of the interactions between  $\alpha$ -helices and their flanking residues.

We hypothesize that the structural similarity of AR-N and the *trp* repressor leads to a corresponding similarity of ligand-induced conformational change. In the absence of tryptophan, an active site arginine residue that had been ligated in a sideways conformation to the  $\alpha$ -carboxylate of the ligand, moves in and occupies the space previously occupied by the tryptophan. The active

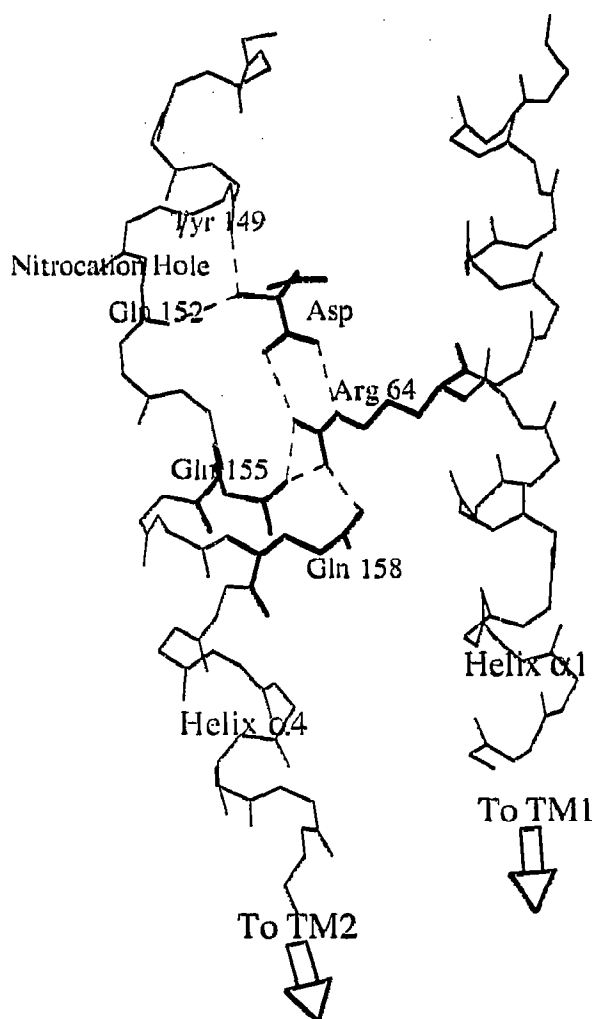


Fig. 4. Interactions of helix  $\alpha 1$ , containing Arg-64, with helix  $\alpha 4$ , containing the nitrification hole. A hydrogen bonding and salt bridge network (dashed line) is centered about Arg-64 in the aspartate receptor. This network connects helices 1 and 4 within a subunit. Note that Arg-64 in helix 1 lies below the nitrification hole in helix 4 by about one turn of an  $\alpha$ -helix.

site arginine and the backbone carbonyls are attached to adjacent  $\alpha$ -helices (and directly flanking residues), and the described motion results in an associated movement of these two helices, relative to one another. We hypothesize that in a similar way aspartate binding causes relative helix-helix conformational changes in the aspartate receptor.

The similarities of AR-N to the *trp* repressor suggest that relative helix-helix motion involving relative  $\alpha 1$  to  $\alpha 4$  movement plays a role in aspartate receptor transmembrane signaling. The positions of Arg-64 in AR-N and Arg-84' in the repressor, relative to their amino acid ligands and nitrocarbon holes, are nearly superimposable. Furthermore, the motion of Arg-84' that occurs in the *trp* repressor seems entirely compatible with the

AR-N structure when the two structures are overlaid (Fig. 3). If AR-N is similar to the *trp* repressor, then in the unliganded state, Arg-64 on  $\alpha 1$  in the aspartate receptor should swing in and interact with the backbone carbonyls in the nitrocarbon hole in  $\alpha 4$ . The distance from the  $\alpha$ -carbon of Arg-64 to the position occupied by the  $\alpha$ -amino nitrogen atom of the aspartate ligand is 9.0 Å. This is too far for the postulated motion to occur without an associated motion of the backbone containing Arg-64, which would translate into a relative motion of helices  $\alpha 1$  and  $\alpha 4$ .

The importance of the hypothesized helix-helix motion in the aspartate receptor becomes evident when one considers the implications of the continuous  $\alpha$ -helices that exist between the aspartate binding site and the transmembrane regions of the receptor. As mentioned, evidence from disulfide cross-linking studies in the transmembrane regions of the aspartate receptor supports a model in which helices  $\alpha 1$  and  $\alpha 4$  at the aspartate binding site continue as unbroken  $\alpha$ -helices through TM1 and TM2 respectively [3,4]. These long  $\alpha$ -helices are expected to act as two rigid rods that could move relative to one another during signaling. A conformational change involving helices  $\alpha 1$  and  $\alpha 4$  could thus propagate from the aspartate-binding site, down through the transmembrane regions to the cytoplasmic signaling domain. Such a relative motion of helices  $\alpha 1$  and  $\alpha 4$  is consistent with the changes observed in the *trp* repressor.

The hypothesized conformational change in the aspartate receptor would involve an intra-subunit change in tertiary structure, as is seen in the *trp* repressor. This does not rule out changes in the quaternary structure of the aspartate receptor caused by ligand binding, but suggests that a tertiary structural change is an important, perhaps major, component of the transmembrane signal. A previous study of hybrid Tar receptors indicated that aspartate-induced increases in methylation could be transmitted through a single subunit of the dimeric receptor, a result that is consistent with the signal being transmitted by changes in tertiary structure [5].

Although we hypothesize that the motions that occur in the ligand-binding sites of the aspartate receptor and the *trp* repressor are similar, we cannot extrapolate from the larger scale tertiary changes in the *trp* repressor to tertiary changes in the aspartate receptor because the tertiary and quaternary structures of AR-N and the *trp* repressor are different. AR-N is a dimer of two compact four-helix bundle subunits which interact along one face of each of the subunits. The *trp* repressor is a dimer of six-helix subunits which are intertwined. The dimer interface of the *trp* repressor is spread throughout the subunit sequences, with all but one helix in each subunit interacting with the opposite subunit in the dimer. Tertiary differences between the two proteins is illustrated by the fact that, when a superposition of

the ligands of the two proteins is performed, the helices containing either Arg-84' (from the *trp* repressor) or Arg-64 (from AR-N) lie essentially perpendicular to each other. The similarity of critical active-site residues between AR-N and the *trp* repressor, combined with a lack of similarity in tertiary and quaternary structure, is reminiscent of the conservation of the residues involved in the charge-relay system in serine proteases [16], assuming that our hypothesis on similarity of function is correct. In this respect, AR-N and the *trp* repressor appear to exhibit homology by convergent evolution in a similar way to that in which the class I mammalian serine proteases share active site similarity, but no sequence or tertiary structural homology with the class II bacterial serine proteases.

*Trp* repressor conformational changes can provide the basis for a model of conformational changes in the binding site of the aspartate receptor, although the tertiary structural differences between the two proteins prevent the associated larger scale conformational changes from being identical. The previously described changes in position of Arg-84' in the *trp* repressor on ligand binding causes its associated  $\alpha$ -helix, helix E', to move relative to helix B from the other subunit (which contains the backbone carbonyls) [11]. Helices E' and B, like helices  $\alpha 1$  and  $\alpha 4$  in the AR-N, are an anti-parallel pair of  $\alpha$ -helices. The helix pairs E-F, and E'-F' make up the helix-turn-helix motifs which bind in the major groove of substrate DNA. The proximity of helix E' to helix B causes the pair of helix-turn-helix structures in the *trp* repressor dimer to be too close to fit in successive major grooves of DNA. The binding of tryptophan, and the accompanying movement of Arg-84' and its associated helix E', moves these structures further apart and creates the correct spacing for DNA binding.

A requirement that the active site in AR-N undergo a conformational change similar to the *trp* repressor would have implications for the resultant tertiary changes in AR-N. Arg-64 in  $\alpha 1$  is positioned somewhat 'below' the level of the nitrocatation hole in  $\alpha 4$  (Fig. 4). Thus it would appear that some relative motion along the long axis of the helices might be required to bring Arg-64 into the nitrocatation hole. Such a motion would correspond to a motion of  $\alpha 4$  relative to  $\alpha 1$  that would probably involve relative motion of these helices up and down, perpendicular to the plane of the membrane. As discussed, a motion of helices  $\alpha 1$  and  $\alpha 4$  should translate into a similar motion of the attached transmembrane regions TM1 and TM2. A motion of a transmembrane helix along its long axis, perpendicular to the plane of the membrane, has been termed a piston motion. Because of the relative rigidity of an  $\alpha$ -helix along its length, motion of a helix along its long axis may be one of the best ways for a modest motion to be transmitted for a long distance. Some local motion of helix  $\alpha 4$  towards helix  $\alpha 1$  could certainly occur as well as the

proposed 'piston' motion. This might result in some rotation of  $\alpha 4$  about its long axis relative to  $\alpha 1$ .

Any relative helix-helix motion in AR-N must be constrained by the interactions between packed helices. In general, when two helices interact they show tight packing of their side-chains, sometimes described as 'ridges into slots' packing [17]. Such tight packing occurs between helices  $\alpha 1$  and  $\alpha 4$  in AR-N. Relative motion between packed helices is constrained by these side-chain interactions. In addition to the *trp* repressor, a number of proteins, including citrate synthase and hemoglobin, show relative helix-helix motion during conformational changes. In this small sample of proteins, relative helix-helix motions of up to 1.5 Å occur without requiring major rearrangements of the side-chains packing the helices together [18]. Because of this pattern, and the general constraint of not rearranging the hydrophobic core of the protein, we believe that a piston motion between  $\alpha 1$  and  $\alpha 4$  is likely to be of the order of 1.5 Å.

A *trp* repressor-based model of AR-N signaling is supported by other aspects of the receptor structure. Of the allosteric enzyme structures that have been solved in different allosteric conformations, the different conformations are usually stabilized by alternating sets of hydrogen bonds and salt bridges [19]. The *trp* repressor model identifies potential sites of alternating sets of hydrogen bonds in the aspartate receptor. Arg-64 in AR-N is the center of a hydrogen bonding and salt bridge network (Fig. 4). Arg-64 hydrogen bonds to two conserved Gln residues, 155 and 158 in  $\alpha 4$ , and also forms a salt bridge to the aspartate ligand which is hydrogen bonded to the backbone carbonyls. In a *trp* repressor-based signaling model, 64 would move in to hydrogen bond to the backbone carbonyls of residues 149 and 152, as well as to the side-chain hydroxyl of Thr-154, in the absence of the aspartate ligand. This motion would break the hydrogen bonds between Arg-64 and the Gln residues. (There are no equivalent residues to Gln-155 and -158 in the *trp* repressor.). In this model one set of hydrogen bonds would be broken and a new set would be formed, analogous to what is seen in allosteric enzymes. These alternate networks of interactions would act to stabilize the alternate conformations of the protein.

The structure of the soluble, N-terminal domain of the aspartate receptor has been solved in both the presence and absence of aspartate, and the conformational changes hypothesized here have not been observed. We hypothesize that the ligand-bound conformation of AR-N is stabilized in the crystal structure, even in the absence of aspartate, because of the presence of a non-native disulfide in AR-N, the fact that AR-N is a fragment lacking both transmembrane regions and C-terminal signaling domains, and/or the very high salt conditions required for crystal formation. One difference that is observed in AR-N between the apo and complex structures is a slight change in the quaternary confor-

mation. This change has been suggested to support a model in which the transmembrane signal in the aspartate receptor is transmitted by a 'scissors'-type motion between the subunits, caused by changes in the quaternary conformation of the dimeric receptor [3]. While such a quaternary change is possibly a component of signaling in the receptor, we believe that it is not the only conformational change involved in signaling.

Our model predicts a key role in signaling for residue Arg-64. Arginine residue 64 in the aspartate receptor is one of three arginine residues (64, 69 and 73) that are conserved among evolutionarily related receptors, and which have been shown by mutagenesis to be crucial for aspartate binding and receptor function. In the crystal structure all three have been identified as interacting with the aspartate ligand [3]. The three binding site arginine residues have been mutated to other residues [20,21]. In these studies, mutations at position Arg-64 were more deleterious to the function of the receptor than mutations at positions 69 and 73, and almost completely eliminated receptor function. This suggests that Arg-64 is especially important in receptor signaling, which is consistent with our hypothesis.

In conclusion, we believe the similarities between the *trp* repressor and AR-N support a *trp* repressor-based model of signaling in the aspartate receptor. The proposed transmembrane signaling intra-subunit conformational change involves the relative motion of two long  $\alpha$ -helices which flank the aspartate binding site and continue through the transmembrane helical regions of the receptor. Continuous helical structures that travel from the binding sites through the transmembrane regions of cell-surface receptors are a logical structural feature which could allow relatively small conformational changes to be transmitted over long distances. We expect that this signaling mode will occur in other receptors.

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